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Oviposition by European Corn Borer (Lepidoptera: Crambidae) in Response to Various Transgenic Corn Events

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ABSTRACT Oviposition preference by European corn borers, *Ostrinia nubilalis* (Hübner), for or against transgenic corn would influence amounts of refuge required for resistance management. The objective of this research was to determine if various *Bacillus thuringiensis* (Bt) corn transgenic events influence *O. nubilalis* oviposition. All commercially available events (currently 5) were evaluated, plus 1 experimental event. Results from 3 independent studies are reported, including 3 field-cage experiments with vegetative corn, 2 field-cage experiments with reproductive corn, and 2 field experiments with natural *O. nubilalis* on reproductive corn. In each case, Bt corn hybrids are compared with their near isogenic hybrids by counting numbers of egg masses on each plant type. More extensive comparisons were made in 3 of the experiments by determining the number, size, and location of egg masses on the corn hybrids. Moths laid more egg masses on Bt corn than on non-Bt corn in 1 cage experiment. These results, however, were not found in any of the other experiments. There is evidence that suggests cage effects influence moth oviposition more than Bt protein. Four of the 5 cage experiments and 2 field experiments indicate that the tested Bt events do not influence *O. nubilalis* oviposition. Larval injury to isogenic corn during the vegetative stage did not influence adult oviposition during the corn reproductive stage when compared with Bt corn and noninjured isogenic corn. Based on these experiments, suggestions are made for future studies that use natural *O. nubilalis* rather than *O. nubilalis* in cages.

KEY WORDS *Ostrinia nubilalis*, *Bacillus thuringiensis*, transgenic corn, refuge, resistance management, oviposition

EUROPEAN CORN BORER, *Ostrinia nubilalis* (Hübner), is a serious pest in the Corn Belt and is second only to the rootworm complex (*Diabrotica* spp.) for numbers of corn hectares treated with insecticides. Untreated *O. nubilalis* larvae cause average losses of 32 bushels per ha in both 1st and 2nd generations in Iowa (Ostlie et al. 1997). Scouting and properly timed applications of chemical or biological insecticides can be effectively used to manage 1st-generation *O. nubilalis* larvae (Moffat 1991). Until *Bacillus thuringiensis* corn was developed, there were no economically effective means for managing the 2nd generation of this pest because insecticide applications are difficult to time because of a prolonged oviposition period (Mason et al. 1996).

Corn hybrids expressing an endotoxin derived from a soil bacterium, *Bacillus thuringiensis* (Berliner) (Bt), were sold commercially for the 1st time in 1996. Bt corn represents a significant departure from traditional control procedures because these plants produce insecticidal proteins throughout the growing season. Dramatic control of *O. nubilalis* (>95%, Koziel et al. 1993) with Bt corn raises concerns about high selection pressure from Bt toxins and subsequent *O. nubilalis* resistance to Bt corn hybrids. Several strategies have been proposed for managing insect adaptation to transgenic plants (Gould 1988a, b, 1989; Raffa 1989). These strategies emphasize the presence of susceptible insects from nontransgenic plants to slow evolution of insect resistance. The premise is that susceptible insects from refuge plants, if present in sufficient numbers, will mate with resistant insects and dilute resistance genes. Any biological factors that influence the number of insects exposed to Bt toxin or influence the production of susceptible insects could substantially impact the refuge strategy. One such factor would be moth oviposition preference. Preference for Bt corn would require more refuge plants to counter an increased selection pressure. However, preference for refuge plants would have the opposite effect. From a resistance management perspective an ideal plant, in addition to killing larvae, would repel adult oviposition. This would reduce selection for resistance because fewer larvae would be exposed to plant toxins.

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Table 1. Bt corn events evaluated in studies conducted in Concord, NE, Ames, IA, and Johnston, IA, in 1994-1997

Event	Bt protein	Gene promoter	Supplier	Trademark	Hybrid	Supplier	Location	Year
MON802	Cry1Ab	CaMV/35S	Monsanto	None	B73xMo17	Public	Ames	1994
176	Cry1Ab	PEPC	Ciba Seeds	None	Experimental	Ciba Seeds	Concord	1994
			Mycogen Seeds	NatureGard	7059	Mycogen Seeds	Ames	1997
			Novartis Seeds	KnockOut	454	Novartis Seeds	Ames	1997
MON810	Cry1Ab	CaMV/35S	Monsanto	YieldGard	35N05/34R06	Pioneer Hi-Bred	Johnston	1996
				YieldGard	34R06	Pioneer Hi-Bred	Ames	1997
BT11	Cry1Ab	CaMV/35S	Monsanto/Novartis	YieldGard	NK7070BT	Novartis Seeds	Ames	1997
DBT418	Cry1Ac	CaMV/35S	DeKalb Genetics	Bt-Xtra	DK580BT	DeKalb Genetics	Ames	1997
CBH351	Cry9C	CaMV/35S	AgrEvo	Starlink[a]	Experimental	Garst Seeds	Ames	1997

[a] CBH351/Starlink was commercially available in 1998.

Oviposition by *O. nubilalis* is influenced by many factors including secondary plant chemicals (Udayagiri and Mason 1995), plant maturity, and plant pubescence (Andrew and Carlson 1976). Plant breeders have used some of these traits to develop corn lines with resistance to *O. nubilalis*. Unfortunately, often these traits are multigenic and difficult to select along with other agronomic traits (Barry and Darrah 1991). Transgenic corn hybrids simplify the breeding process for *O. nubilalis* resistance because the exotic DNA can be a single gene, and inheritance is dominant because there is no complementary allele. Bt DNA is inserted into corn DNA through various transformation techniques. Successful transformations, called "events," are carefully screened for Bt production and agronomic traits. Each event is unique and could alter physiological pathways, physical structures, or developmental processes that influence moth behavior.

The 1st study to compare *O. nubilalis* oviposition preference for a non-Bt corn hybrid or its Bt counterpart suggests that moths are not influenced by *B. thuringiensis* Cry1Ab endotoxin (Orr and Landis 1997). This supports earlier studies that indicate oviposition of diamondback moth, *Plutella xyostella* (L.), females on cabbage leaf disks in petri dishes (Groeters et al. 1992) and broccoli plants (Riggin-Bucci and Gould 1996) is not affected by the presence of *B. thuringiensis* toxins. Orr and Landis (1997) evaluated a single 176-event hybrid. Now, 5 Bt events are commercially available in multiple corn hybrids that express Cry1Ab, Cry1Ac, or Cry9C proteins.

The objective of this research was to determine if various Bt events influence the number of eggs that *O. nubilalis* oviposits on corn hybrids. This work expands on the Orr and Landis (1997) 176-event study by including additional 176-event hybrids, 3 other Cry1Ab events (MON802, MON810, and BT11), a Cry1Ac (DBT418) event, and a Cry9C event (CBH351). Results from independent studies conducted at 3 locations are reported.

Materials and Methods

***Bacillus thuringiensis* Corn Hybrids.** Hybrids used in these studies are listed in Table 1. Endotoxins expressed in the transgenic plants include Cry1Ab, Cry1Ac (both derived from *B. thuringiensis* subsp. *kurstaki*), and Cry9C (derived from *B. thuringiensis* subsp. *tolworthi*). The Bt hybrids have 2 expression profiles based on gene promoters. Plants with the cauliflower mosaic virus (CaMV) 35S promoter express endotoxins throughout the entire plant, whereas those with the combination of the maize phosphoenolpyruvate carboxylase (PEPC) and a maize pollen specific promoter express in green tissue and pollen (Koziel et al. 1993). Bt events used in the 1994 studies were put into experimental corn hybrids. Commercial hybrids were available for Bt events 176, Bt11, MON810, and DBT418 in 1997, and CBH351 became commercially available in 1998 (EPA 1998).

Although the following experiments were started independently, they share common experimental procedures. Bt corn varieties were compared with their near isogenic varieties. Row spacings were 0.76 m, and plant densities were ≈50,000 plants per hectare. Fields were prepared with conventional tillage, N 140–200 kg/ha, and preemergence herbicides. Moths used in the cage experiments were from the 6–8th generation of laboratory-reared colonies established the previous summers from feral *O. nubilalis* in Iowa. Moths were reared using the methods described by Guthrie (1987). Egg masses on corn were circled with a waterproof marker to prevent recounting during repeated observations. Both vegetative and reproductive growth stages of corn were evaluated. Nomenclature from Ritchie et al. (1997) was used to designate plant growth stages, where Vn = vegetative n-leaf stage, R1 = reproductive silking stage, R2 = reproductive blister stage, and R3 = reproductive milk stage.

Corn hybrids with the 176 event (V8 and R1) were evaluated in cage experiments near Concord, NE, in 1994. A hybrid containing Bt event MON802 was evaluated in 1994 near Ames, IA, in a cage study (V12) and in a field study (R1 and R3). Two hybrids containing Bt event MON810 were evaluated in 1996 in cage studies (V8 and R1) near Johnston, IA. Commercial hybrids with events 176, Bt 11, MON810, DBT418, and CBH351 were evaluated in 1997 during a field study (R2) near Ames, IA.

Concord, NE, Experiments. *Vegetative Corn.* Cage experiments were conducted at the University of Nebraska Haskell Agricultural Laboratory located 2.5 km east of Concord, NE. Alternating double rows (3 m long) of Bt corn (176 in Ciba experimental hybrid) and non-Bt near isogenic corn (Ciba experimental)

were planted 11 May 1994. Oats (variety Ogle), which later served as aggregation sites for the moths, were planted in between the rows on the same day. Twelve saran (1.8 m^3) cages were put over paired rows (randomly assigned: 1 row Bt, 1 row non-Bt) of corn on 9 June. Plants were thinned to 7 plants per row. There were 2 sets of 6 cages: the 1st set was used to test vegetative stage corn, and the 2nd set was used to test reproductive stage corn. Twenty moths (2 d old;10 male, 10 female) were introduced into each of 6 cages on 17 June. Corn developmental stage was V8. Egg masses on each plant were counted on 20, 23, 27, and 29 June, and 1 July.

Reproductive Corn. Moths were introduced into the 2nd set of 6 cages on 18 July. Corn developmental stage was R1. Egg masses on each plant were counted on 21 and 25 July, and 2 and 5 August.

Johnston, IA, Experiments. *Vegetative Corn.* Two MON810 Bt hybrids that expressed high levels of Cry1Ab protein [≈1,200 pg/μg (Harlow and Lane 1988), V6 leaf] paired with non-Bt near isogenic hybrids were evaluated in 1996. The hybrids were planted 17 May 1996 on the Pioneer Hi-Bred Research Farm, Johnston, IA. Each Bt hybrid and its paired non-Bt hybrid were replicated 4 times for vegetative stage corn and 3 times for reproductive stage corn. Plots were 5.3 m long. Field cages (1.8 by 3 by 3 m) were placed over paired rows of hybrid corn (randomly assigned: 1 row Bt, 1 row non-Bt) at growth stage V8. Field cages were constructed of aluminum pipe frames covered with Lumite (18 by 14 mesh, Divison of Synthetic Industries, Gainesville, GA) anchored at the base with tent stakes. Cage-covered plants were thinned to equal numbers, ≈10 plants per row. One *O. nubilalis* pupation ring (Guthrie 1987) from the laboratory colony was hung in the middle of each cage from a wire hook ≈40 cm from the ground. Each pupation ring contained ≈300 pupae with an estimated 50:50 sex ratio. Adults emerged in 3–4 d. Ten days after emergence, egg masses were counted on each plant in the cage.

Reproductive Corn. R1 corn was evaluated in cages with procedures similar to those stated for V8 corn. Cages were erected at anthesis, and moth rings were put into the cages the following day. Ten days after emergence, egg masses were counted on 10 plants per row in each cage.

Ames, IA, Experiments. *Vegetative Corn.* Cage experiments were conducted at the Iowa State University Woodruff farm located 6 km southwest of Ames, IA. Alternating double rows (27.4 m long) of Bt corn (MON802) and non-Bt near isogenic corn (B73× Mo17) were planted 23 May 1994. Sixteen Lumite (18 by 14 mesh) cages (1.8 m^3) were put over 2 rows of corn in 4 arrangements on 8 July, choice: (1) Bt/non-Bt corn hybrids with Bt corn row on the south side of the cage, (2) Bt/non-Bt corn hybrids with Bt corn row on the north side of the cage, and no choice, (3) 2 rows of Bt corn, and (4) 2 rows of non-Bt corn. Planting arrangements were assigned randomly within 4 blocks. Plants within each cage were thinned to 6 per row. Thirty moths (2 d old; 15 male, 15 female) were introduced into each of the cages the evening of 11 July. Corn developmental stage was V12. Number of egg masses and location of each egg mass (leaf number; position on leaf divided into quartiles by length, e.g., 1/4 = leaf quartile closest to stalk; and side of leaf) on each plant were determined on 12, 13, and 14 July.

Reproductive Corn. Field experiments with natural *O. nubilalis* were conducted at the Iowa State University Woodruff farm. Four-row plots (9.1 m long) of Bt corn (MON802) and non-Bt corn (B73× Mo17) were planted 14 May 1994. These were replicated 12 times. Number and location of egg masses, as previously described, were observed on 10 randomly selected plants from each of the 24 plots on 27 July, during the 2nd *O. nubilalis* flight. The developmental stage of the plants was R1. In addition, location of egg masses (leaf number, leaf quartile, and side of leaf) and number of eggs per mass were determined for 6 Bt and 6 non-Bt plots on 18 August, during the end of the 2nd *O. nubilalis* flight, when corn stage was R3. Ten egg masses were evaluated per plot.

All Commercial Events. Field experiments with natural *O. nubilalis* also were conducted at the Iowa State University Bruner farm located 10 km west of Ames, IA. Seven varieties of Bt corn hybrids (176 Novartis Seeds, 176 Mycogen Seeds, MON810, Bt11, DBT418, and CBH351) and their near isogenic hybrids were planted in 4-row plots (2 m long) arranged in 10 randomized complete blocks on 20 May 1997 (Table 1). One of the outside rows of each plot was manually infested with ≈50 *O. nubilalis* neonates 7 July. Number of egg masses and location of each egg mass on each plant were determined for 2 plants selected from manually infested rows and 2 plants selected from uninfested rows 8, 12, and 13 August during the 2nd *O. nubilalis* flight. Leaf number was assigned relative to the ear leaf (e.g., leaf below ear = −1, ear leaf = 0, leaf above ear = 1). Numbers of eggs in the 1st and 6th egg mass on each plant were counted. The developmental stage of the plants was R2. Non-Bt plants that had not been manually infested were not evaluated if they had visible signs of *O. nubilalis* injury. Such plants were rare because as a result of the late planting date these plants were not attractive to 1st-generation moths. None of the Bt plants had visible signs of *O. nubilalis* injury even though some of them were manually infested.

Data Analyses. Number of egg masses on Bt and non-Bt vegetative and reproductive corn in the Concord, NE, cage experiments were each analyzed with two-way analysis of variance (ANOVA) with repeated measures, where corn type and cage were grouping factors, and sampling date was the repeated measure (SAS Institute 1985). Similar data collected in Johnston, IA, were analyzed with three-way ANOVA, where corn type, hybrid, and block were grouping factors. Number of egg masses and location of egg masses (leaf number, leaf quartile, and side of leaf) on vegetative corn in the 1994 cage experiment in Ames, IA, each were analyzed with a split-plot ANOVA with repeated measure. Choice and block were the main plots, corn type (Bt, non-Bt hybrids) and row direc-

Table 2. Mean ± SEM number of *O. nubilalis* egg masses per cage on non-Bt and BT corn (non-Bt experimental hybrid and 176-event near isogenic hybrid) in cage experiments during corn vegetative and reproductive stages in Concord, NE, in 1994

Stage of corn development	Date	Non-Bt corn	Bt corn
V8[a]	20 June	5.2 ± 1.08	6.8 ± 1.28
	23 June	5.5 ± 1.18	5.8 ± 1.28
	27 June	3.3 ± 1.41	6.5 ± 1.73
	29 June	5.2 ± 0.48	5.3 ± 0.33
	1 July	2.8 ± 0.91	3.7 ± 0.99
R1[b]	21 July	2.7 ± 1.67	3.2 ± 1.14
	25 July	7.5 ± 2.47	7.2 ± 2.75
	29 July	7.2 ± 0.40	5.2 ± 1.14
	2 Aug.	1.2 ± 0.79	1.5 ± 0.50
	5 Aug.	0.5 ± 0.34	1.3 ± 0.61

[a] There are significant plant-type ($F = 9.14$; df = 1, 5; $P \leq 0.05$) and cage ($F = 6.83$; df = 5, 5; $P \leq 0.05$) effects. Sampling date is an important factor ($F = 3.70$; df = 4, 20; $P = 0.052$); date by corn-type interaction ($F = 1.22$; df = 4, 20; $P = 0.3364$) is not significant, but the date by cage interaction ($F = 11.2$; df = 20, 20; $P \leq 0.05$) is significant.

[b] Plant-type ($F = 0.09$; df = 1, 5; $P = 0.78$) effect is not significant, but cage ($F = 11.8$; df = 5, 5; $P \leq 0.01$) and sampling date ($F = 24$, 6; df = 4, 20; $P \leq 0.01$) effects are significant. Date by corn-type interaction ($F = 0.94$; df = 4, 20; $P = 0.40$) is not significant, but the date by cage interaction ($F = 4.17$; df = 20, 20; $P \leq 0.05$) is significant.

tion (north and south) were subplots, and sampling date was the repeated measure. Number of natural *O. nubilalis* egg masses and location of egg masses, as described above, on reproductive corn in Ames, IA, 1994 each were analyzed with three-way ANOVA, where corn type, corn stage, and block were grouping factors. Number of egg masses on corn and location of egg masses in the experiment using 5 commercial events in Iowa in 1997 each were analyzed with a split-plot ANOVA. Hybrid and block were main plots and treatment (Bt hybrid not injured, non-Bt hybrid injured, and non-Bt hybrid not injured) was the subplot. Waller–Duncan *K*-ratio *t*-tests (Waller and Duncan 1969) were used to separate means when significant effects were found. The Greenhouse–Geisser procedure was used to adjust *P* to account for consecutive-day dependence when repeated measures was used (Winer 1962).

Results and Discussion

Concord, NE. *Vegetative Corn.* More *O. nubilalis* egg masses were found on Bt corn than on non-Bt corn in the Concord cage experiment (event 176, V8) (Table 2). Cage effect was significant, and sampling date was an important factor. The date by corn-type interaction was not significant, but the date by cage interaction was significant. It was surprising that more eggs were laid on the Bt corn, but the cage and sampling-date effects and the date by cage interaction suggest that variables other than plant type (e.g., shading and weather) could have affected oviposition.

Reproductive Corn. Number of *O. nubilalis* egg masses on Bt and non-Bt corn did not differ in the 2nd cage experiment (event 176, R1) (Table 2). Cage, sampling date, and date by cage interaction again were important factors at this location. The date by corn-type interaction was not significant.

Johnston, IA. *Vegetative and Reproductive Corn.* Number of egg masses on Bt and non-Bt corn did not differ in either of the Johnston cage experiments (event MON810, V8) (Table 3). Hybrid effect also was not significant.

Ames, IA. *Vegetative Corn.* Number of egg masses on corn hybrids did not differ under choice and no-choice conditions in the Ames cage experiment (event MON802, V12) (Table 4). Corn type and direction effects, and corn-type by choice interaction also were not significant. Lack of a choice by corn-type interaction suggests that moths laid similar numbers of egg masses on Bt and non-Bt corn under choice and no-choice conditions. There was a significant date effect, and number of egg masses on corn varied over date depending on the direction of rows within cages. The date by choice, date by corn-type, and date by choice by corn-type interactions were not significant. The significant date by row-direction interaction could be the result of cage effects. Shading, wind, and other factors could have influenced moth behavior in cages.

Placement of *O. nubilalis* egg masses on corn hybrids did not differ under choice and no-choice conditions in the Ames cage experiment (event MON802, V12) (Table 5). Corn-type and row-direction effects, and choice by corn-type interaction also were not significant. These results suggest that placement of egg masses on the plants was not influenced by Bt or non-Bt corn under choice and no-choice conditions. Sampling date was an important factor for leaf number, which could be the result of a significant date by block interaction. Date by choice, date by corn-type, date by direction, and date by choice by corn-type interactions were not significant. These results suggest that placement of egg masses on leaves was not influenced by Bt or non-Bt corn under choice and no-choice conditions. Sampling date was not an important factor for leave quartile or side of leaf.

Reproductive Corn. Number of natural *O. nubilalis* egg masses on Bt and non-Bt corn did not differ (mean ± SEM: Bt corn 3.4 ± 0.28; non-Bt corn 3.5 ± 0.28; $F = 0.06$; df = 1, 11; $P = 0.81$) in the Ames field experiment (event MON802, R1). Additionally, the number of eggs per mass during R3 inspections were not significantly different (mean ± SEM: Bt corn 19.4 ± 0.71; non-Bt corn 19.8 ± 0.83; $F = 0.49$; df = 1, 5; $P = 0.51$).

Table 3. Mean ± SEM number of *O. nubilalis* egg masses on non-Bt corn hybrids 3563 and 3489 and Bt corn hybrids 35N05 and 34R06 (MON810 event) in cage experiments during corn vegetative and reproductive stages in Johnston, IA, in 1996

Stage of corn development	Hybrids	Non-Bt corn	Bt corn
V8[a]	3563/35N05	6.3 ± 1.92	5.7 ± 0.62
	3489/34R06	6.8 ± 0.78	5.7 ± 0.59
R1[b]	3563/35N05	6.1 ± 0.78	5.7 ± 0.73
	3489/34R06	5.8 ± 1.21	6.7 ± 2.05

[a] There are no significant plant-type ($F = 0.61$; df = 1, 10; $P = 0.45$) and hybrid ($F = 0.05$; df = 1, 10; $P = 0.83$) effects.

[b] There are no significant plant-type ($F = 0.10$; df = 1, 7; $P = 0.76$) and hybrid ($F = 0.13$; df = 1, 7; $P = 0.73$) effects.

Table 4. Mean ±SEM number of *O. nubilalis* egg masses per plant on V12 stage corn in cage experiments in Ames, IA, 1994

Date	Choice		No Choice		Row Direction[a]	
	Non-Bt corn[b]	Bt corn[c]	Non-Bt corn	Bt corn	North	South
12 July	2.0 ± 0.71	1.5 ± 0.51	2.0 ± 0.68	2.0 ± 0.61	1.6 ± 0.39	2.2 ± 0.47
13 July	1.1 ± 0.36	1.3 ± 0.30	1.7 ± 0.45	1.6 ± 0.38	1.5 ± 0.19	1.4 ± 0.32
14 July	2.8 ± 0.77	2.9 ± 0.66	3.2 ± 0.74	3.4 ± 0.43	3.8 ± 0.49	2.4 ± 0.34

Choice ($F = 5.08$; df = 1, 3; $P = 0.11$), corn type ($F = 0.0003$; df = 1, 20; $P = 0.99$), and direction ($F = 2.02$; df = 1, 20; $P = 0.17$) effects, and corn-type by choice interaction ($F = 0.06$; df = 1, 20; $P = 0.81$) are not significant. Sampling date ($F = 27.9$; df = 2, 40; $P \leq 0.01$) and date by direction interaction ($F = 10.1$; df = 2, 40; $P \leq 0.01$) are significant. Date by choice ($F = 0.12$; df = 2, 40; $P = 0.86$), date by corn-type ($F = 0.40$; df = 2, 40; $P = 0.64$), and date by choice by corn-type ($F = 0.52$; df = 2, 40; $P = 0.57$) interactons are not significant.

[a] Rows were planted east to west within cages.

[b] B73xMo17 hybrid.

[c] MON802 Bt event in B73xMo17 genetic background.

Placement (leaf number, leaf quartile, and side of leaf) of natural *O. nubilalis* egg masses on Bt and non-Bt corn hybrids did not differ in the Ames field experiment (R1 and R3), nor was the corn-development effect significant for the 3 measures (Table 6).

All Commercial Events. The previous experiments suggest that cage effects potentially influence moth oviposition more than Bt protein. This last experiment was conducted in corn fields with natural *O. nubilalis* to minimize possible experimental artifacts.

Number of *O. nubilalis* egg masses on the 3 treatments (Bt hybrid not injured, non-Bt hybrid injured, and non-Bt hybrid not injured) did not differ in the Ames field experiment (R2), nor were the hybrid effects significant (Table 7). The treatment by hybrid interaction also was not significant, which shows treatment differences within the hybrids did not occur.

The number of *O. nubilalis* eggs per mass on the corn-type-injury treatments did not differ, but there was a significant hybrid effect (Table 7). The treatment by hybrid interaction was not significant. This hybrid effect could be related to a number of factors, e.g., plant pubescence could differ among the hybrids (Andrew and Carlson 1976).

Placement (leaf number, leaf quartile, and side of leaf) of *O. nubilalis* egg masses on the corn-type-injury treatments in the Ames field experiment were not significantly different (Table 7). Hybrid effects and treatment by hybrid interactions for the 3 measures were not significant.

The oviposition result at the Concord location for vegetative corn in cages, where more eggs were found on Bt corn than on non-Bt corn, was not confirmed with the other experiments. There was evidence, however, that suggests cage effects could influence moth oviposition more than Bt protein. Four of the 5 cage experiments and both of the field experiments confirm previous results showing that *O. nubilalis* oviposition is not influenced by Bt proteins. This study goes a step further and suggests that none of the commercially available or experimental corn events tested in these studies influence *O. nubilalis* oviposition.

None of the *O. nubilalis* injury sustained during the corn vegetative stage was severe enough to influence *O. nubilalis* oviposition during the corn reproductive stage. Differential oviposition in this case would have been an indirect effect of Bt corn because little to no vegetative *O. nubilalis* injury is found in commercially

Table 5. Location of *O. nubilalis* egg masses found on non-Bt and Bt corn in cage experiment (MON802, V12) in Ames, IA, 1994 (mean ± SEM leaf number, leaf quartile, and proportion of eggs laid under leaves)

Observation	Date	Choice		No Choice	
		Non-Bt corn	Bt corn	Non-Bt corn	Bt corn
Leaf no.[a]	12 July	8.8 ± 0.25	9.1 ± 0.41	9.1 ± 0.52	9.6 ± 0.22
	13 July	9.2 ± 0.55	9.8 ± 0.46	9.5 ± 0.33	9.1 ± 0.30
	14 July	9.4 ± 0.45	9.9 ± 0.37	9.9 ± 0.51	9.7 ± 0.40
Location on leaf (quartile)[b]	12 July	0.74 ± 0.07	0.64 ± 0.06	0.54 ± 0.04	0.64 ± 0.05
	13 July	0.58 ± 0.06	0.66 ± 0.04	0.62 ± 0.05	0.66 ± 0.06
	14 July	0.59 ± 0.04	0.64 ± 0.02	0.61 ± 0.05	0.58 ± 0.05
Proportion under leaves[c]	12 July	0.45 ± 0.13	0.40 ± 0.13	0.46 ± 0.10	0.42 ± 0.10
	13 July	0.50 ± 0.10	0.47 ± 0.09	0.61 ± 0.11	0.61 ± 0.08
	14 July	0.59 ± 0.04	0.45 ± 0.07	0.47 ± 0.08	0.57 ± 0.05

[a] There are no significant choice ($F = 0.07$; df = 1, 3; $P = 0.82$), corn-type ($F = 1.89$; df = 1, 15; $P = 0.19$), and direction ($F = 0.09$; df = 1, 15; $P = 0.36$) effects, nor are the choice by corn-type ($F = 1.87$; df = 1, 15; $P = 0.19$) and corn-type by direction ($F = 0.11$; df = 1, 15; $P = 0.74$) interactions significant. Sampling date ($F = 5.27$; df = 2, 30; $P \leq 0.05$) and the date by block interaction ($F = 3.77$; df = 6, 30; $P \leq 0.05$) are significant, but the date by choice ($F = 1.83$; df = 2, 30; $P = 0.19$), date by corn-type ($F = 0.25$; df = 2, 30; $P = 0.73$), date by direction ($F = 1.44$; df = 2, 30; $P = 0.25$), and date by choice by corn-type ($F = 3.03$; df = 2, 30; $P = 0.08$) interactions are not significant.

[b] There are no significant choice ($F = 0.98$; df = 1, 3; $P = 0.43$), corn-type ($F = 0.35$; df = 1, 15; $P = 0.56$), and direction ($F = 0.81$; df = 1, 15; $P = 0.38$) effects, nor is the choice by corn-type interaction ($F = 0.09$; df = 1, 15; $P = 0.77$) significant. Sampling date ($F = 0.42$; df = 2, 30; $P = 0.66$) is not an important factor.

[c] There are no significant choice ($F = 0.74$; df = 1, 3; $P = 0.48$), corn-type ($F = 0.02$; df = 1, 15; $P = 0.90$), and direction ($F = 0.15$; df = 1, 15; $P = 0.70$) effects, nor is the choice by corn-type interaction ($F = 0.35$; df = 1, 15; $P = 0.56$) significant. Sampling date ($F = 0.58$; df = 2, 30; $P = 0.56$) is not an important factor.

Table 6. Location of natural *O. nubilalis* egg masses found on non-BT and Bt field corn (MON802, R1 and R3) in Ames, IA, 1994

Observation	Stage of corn development	Non-Bt corn	Bt corn
Leaf no.[a]	R1	12.6 ± 0.13	12.5 ± 0.13
	R3	12.1 ± 0.24	12.3 ± 0.25
Location on leaf (quartile)[b]	R1	0.53 ± 0.01	0.52 ± 0.02
	R3	0.47 ± 0.02	0.48 ± 0.04
Proportion under leaves[c]	R1	0.75 ± 0.02	0.73 ± 0.03
	R3	0.73 ± 0.05	0.79 ± 0.06

[a] There are no significant corn-type ($F = 1.42$; df = 1, 22; $P = 0.25$) and corn-development ($F = 0.02$; df = 1, 22; $P = 0.90$) effects.
[b] There are no significant corn-type ($F = 0.01$; df = 1, 22; $P = 0.94$) and corn-development ($F = 3.87$; df = 1, 22; $P = 0.06$) effects.
[c] There are no significant corn-type ($F = 0.001$; df = 1, 22; $P = 0.97$) and corn-development ($F = 0.004$; df = 1, 22; $P = 0.95$) effects.

available Bt hybrids. Preference by *O. nubilalis* for noninjured plants during the 2nd moth flight would increase oviposition, and thus selection pressure, on transgenic plants. These results, however, could be different for less tolerant varieties of corn, such as popcorn, sweet corn, and perhaps other varieties of field corn.

Corn breeders should not ignore the possibility that current events in other genetic backgrounds or future transgenic events could influence *O. nubilalis* oviposition. Based on experience from these studies we recommend that future studies be conducted with natural populations of *O. nubilalis*. Such studies are less labor intensive than cage studies and avoid possible cage effects. Corn plants that deter or attract *O. nubilalis* oviposition could be useful for developing new resistance management strategies or for developing the next generation of transgenic plants. Plants selected for high and low tendencies to attract moth oviposition could provide valuable clues about chemical pathways associated with plant protection mechanisms. Selection pressure for resistant insects would be reduced if insecticidal genes such as Bt were coupled with genes modified for ovipositional deterrence.

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Table 7. Mean ± SEM number and location of *O. nubilalis* egg masses found on Bt and non-Bt corn plants during anthesis in Ames, IA, 1997

Hybrid[a]	(Bt Treatment Event)	*O. nubilalis* injury	No. of egg masses[b]	No. of eggs per egg mass[c]	Egg mass location		
					Proximity to ear[d]	Location on leaf[e]	Proportion under leaf[f]
1	Yes (176)	No	2.8 ± 0.4	14.6 ± 0.8	0.8 ± 0.5	0.48 ± 0.04	0.63 ± 0.11
1	No	Yes	3.0 ± 0.7	14.3 ± 1.9	0.1 ± 0.7	0.54 ± 0.05	0.65 ± 0.11
1	No	No	2.2 ± 0.6	17.5 ± 2.2	−0.5 ± 1.0	0.37 ± 0.04	0.79 ± 0.11
2	Yes (DBT418)	No	2.3 ± 0.5	15.1 ± 1.6	0.2 ± 0.7	0.57 ± 0.07	0.74 ± 0.08
2	No	Yes	2.2 ± 0.6	17.8 ± 2.8	−0.1 ± 0.8	0.51 ± 0.06	0.57 ± 0.16
2	No	No	2.4 ± 0.6	16.6 ± 2.1	1.7 ± 0.7	0.63 ± 0.06	0.57 ± 0.12
3	Yes (MON810)	No	2.9 ± 0.7	14.1 ± 2.4	0.8 ± 0.9	0.52 ± 0.06	0.48 ± 0.08
3	No	Yes	1.7 ± 0.4	15.6 ± 1.9	0.2 ± 0.4	0.58 ± 0.05	0.68 ± 0.12
3	No	No	1.7 ± 0.5	17.4 ± 2.5	0.6 ± 0.8	0.59 ± 0.09	0.71 ± 0.15
4	Yes (BT11)	No	2.7 ± 0.6	19.5 ± 3.0	−0.4 ± 0.6	0.46 ± 0.04	0.89 ± 0.08
4	No	Yes	3.2 ± 0.9	28.9 ± 4.2	−0.6 ± 0.4	0.62 ± 0.06	0.84 ± 0.11
4	No	No	4.2 ± 1.1	21.6 ± 3.7	−0.3 ± 0.9	0.58 ± 0.06	0.65 ± 0.12
5	Yes (CBH351)	No	2.3 ± 0.6	19.7 ± 2.8	−0.1 ± 0.7	0.48 ± 0.06	0.93 ± 0.05
5	No	Yes	2.2 ± 0.5	17.9 ± 1.7	0.2 ± 0.6	0.59 ± 0.08	0.91 ± 0.07
5	No	No	2.2 ± 0.6	20.0 ± 4.0	1.0 ± 0.6	0.63 ± 0.08	0.77 ± 0.11
6	Yes (176)	No	3.1 ± 0.8	22.3 ± 3.2	0.1 ± 0.7	0.60 ± 0.04	0.76 ± 0.06
6	No	Yes	2.8 ± 0.4	20.4 ± 2.3	1.1 ± 0.4	0.56 ± 0.04	0.69 ± 0.06
6	No	No	2.8 ± 0.9	17.0 ± 3.1	1.0 ± 0.7	0.64 ± 0.09	0.84 ± 0.10

[a] Hybrid 1 pair: Ciba 4494, Ciba Maximizer 454; hybrid 2 pair: DEKALB DK580, DEKALB DK580BT; hybrid pair 3: Pioneer Hi-Bred 3489, Pioneer Hi-Bred 34R06; hybrid pair 4: Northrup King NK7070, Northrup King NK7070BT; hybrid pair 5: Garst experimental, Garst experimental Bt hybrid; hybrid pair 6: Mycogen 7050bc, Mycogen NG7059.
[b] There are no significant treatment ($F = 0.15$; df = 2, 87; $P = 0.86$) and hybrid ($F = 1.25$; df = 5, 45; $P = 0.30$) effects, nor is the treatment by hybrid interaction ($F = 0.78$; df = 10, 87; $P = 0.65$) significant.
[c] There is no significant treatment ($F = 0.50$; df = 2, 64; $P = 0.61$) effect, but there is a significant hybrid effect [hyrid pairs (HP), mean ± SEM: HP4 22.8 ± 2.2a, HP6 19.6 ± 1.7ab, HP5 19.6 ± 1.8ab, HP2 16.5 ± 1.3b, HP1 15.5 ± 1.1b, HP3 15.0 ± 1.2b] ($F = 2.9$; df = 5, 43; $P \leq 0.05$); the treatment by hybrid interaction ($F = 0.93$; df = 10, 69; $P = 0.51$) is not significant. Means followed by the same letter are not significantly different (Waller–Duncan, $P \leq 0.05$).
[d] There are no significant treatment ($F = 0.68$; df = 2, 69; $P = 0.51$) and hybrid ($F = 1.10$; df = 5, 43; $P = 0.37$) effects, nor is the treatment by hybrid interaction ($F = 1.08$; df = 10, 69; $P = 0.39$) significant.
[e] There are no significant treatment ($F = 2.66$; df = 2, 69; $P = 0.08$) and hybrid ($F = 0.24$; df = 5, 43; $P = 0.95$) effects, nor is the treatment by hybrid interaction ($F = 0.84$; df = 10, 69; $P = 0.59$) significant.
[f] There are no significant treatment ($F = 0.31$; df = 2, 69; $P = 0.73$) and hybrid ($F = 2.19$; df = 5, 43; $P = 0.07$) effects, nor is the treatment by hybrid interaction ($F = 1.46$; df = 10, 69; $P = 0.17$) significant.

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